Rapid communications

The cataract Shionogi mouse, a sister strain of the non-obese diabetic mouse: similar class II but different class I gene products

H. Ikegami¹, S. Makino², M. Harada³, G. S. Eisenbarth¹ and M. Hattori¹

¹ The Research Division, Joslin Diabetes Center, Boston, Massachusetts, USA

² Aburahi Laboratories, Shionogi and Company Ltd, Shiga,

³ Shionogi Research Laboratories, Shionogi and Company Ltd, Osaka, Japan

Summary. We have studied with a series of monoclonal antibodies and restriction fragment analysis the K, D, and class II region of the major histocompatibility complex of the non-obese diabetic mouse in comparison with its sister strains, the non-obese non-diabetic and cataract Shionogi mouse. (1) K region: Monoclonal antibody 31-3-4S (anti-K^d) reacted with splenocytes from non-obese diabetic mice while other anti-K (K^b, K^k, K^q) monoclonals did not react. Splenocytes from non-obese non-diabetic mice reacted with both anti-K^b and K^k monoclonals while splenocytes from cataract Shionogi mice reacted with anti-K^d and K^k monoclonals. Both sister strains, therefore, differ from the non-obese diabetic and other known mice strains by monoclonal analysis of H-2K. (2) D region: Splenocytes from both non-obese diabetic and non-obese non-diabetic mice reacted with monoclonal antibody 28-14-8S (anti-D^b) while splenocytes from cataract Shionogi mice did not react with any anti-D monoclonal antibody tested. (3a) Class II region (non-obese diabetic and non-obese non-diabetic mice): Three of 11 monoclonal antibodies to class II molecules reacted with splenocytes of the non-obese diabetic mouse. The 3 reacting monoclonals have I-A^k primary specificities though additional anti-I-A^k monoclonal antibodies were negative. Among these monoclonals, 39B and 40A reacted with the non-obese diabetic mouse but not with the non-obese non-diabetic mouse, while 10-2-16 reacted with non-obese diabetic, non-obese non-diabetic and cataract Shionogi mice. Monoclonal MKD6 (anti-I-A^d) reacted with non-obese non-diabetic but not non-obese diabetic mice. In crosses of non-

obese diabetic with non-obese non-diabetic mice, splenocytes from all diabetic backcrosses studied (6/6) were positive with monoclonal 40A but negative with MKD6 indicating that the major histocompatibility complex of non-obese non-diabetic mice is not diabetogenic and confirming major histocompatibility complex-linkage of the diabetogenic gene in this additional cross. (3b) Class II region (cataract Shionogi mice): Utilising 11 anti-class II monoclonal antibodies the pattern of reactivity of the cataract Shionogi mouse was identical to the non-obese diabetic mouse. Splenocytes from both non-obese diabetic and cataract Shionogi mice fail to express I-E (no reaction with monoclonal 14-4-4). In addition, using an I-A alpha probe with restriction endonuclease HindIII or I-A beta probe with BamHI, the restriction fragment length polymorphism pattern of the cataract Shionogi mouse was identical to the non-obese diabetic mouse. In summary, the cataract Shionogi mouse appears to have a similar I-A region to the non-obese diabetic mouse but differs at both the K and D region. With the hypothesis that the unique I-A beta of the non-obese diabetic mouse is diabetogenic, the cataract Shionogi mouse should provide a new strain for further characterisation of diabetogenic genes of the non-obese diabetic mouse since it is similar at I-A, fails to express I-E, but differs at both class I loci.

Key words: Major histocompatibility complex, non-obese diabetic mouse, non-obese non-diabetic mouse, cataract Shionogi mouse, insulin-dependent diabetes, restriction fragment length polymorphisms.

The non-obese diabetic (NOD) mouse spontaneously develops insulin-dependent diabetes mellitus with massive lymphocytic infiltration into the pancreatic islets [1]. Approximately 80% of females and 20% of males develop overt diabetes at 30 weeks of age [1]. Our prior breeding study in crosses of NOD mice with control C3H mice indicated that two or more recessive diabetogenic genes determine diabetes susceptibility and at least one of them is linked to the major histo-

compatibility complex (MHC) of the NOD mouse [2, 3]. This observation was recently confirmed by other laboratories [4, 5]. Therefore, it is of great importance to characterise the MHC of the NOD mouse in comparison with its related strains in order to analyse and localise the diabetogenic gene(s) in the MHC.

The NOD mouse was originally derived from outbred Imperial Cancer Research (ICR) mice [1]. One mouse with cataracts and small eyes was found in

Fig. 1. Genealogy of the non-obese diabetic (NOD), non-obese non-diabetic (NON) and cataract Shionogi (CTS) mouse. Their types of major histocompatibility gene products are summarised on the right. * Unique reactivity, reacting with both anti-K^d and -K^k monoclonal antibodies. ** Unique reactivity for the NOD mouse, not fitting any known specificity pattern. Tentatively named as "nod" type. *** Not reacting with the monoclonal antibodies studied (b,d,k,q), and may be unique for the CTS mouse. Tentatively named as "cts" type. **** Unique reactivity, reacting with both anti-K^b and -K^k monoclonal antibodies. ***** Unique reactivity for the NON mouse, not fitting any known specificity pattern. Tentatively named as "non" type

outbred ICR mice and an inbred cataract Shionogi (CTS) strain characterised by cataracts and microphthalmia was established [6]. Since cataracts are often observed in diabetic patients, two sublines were separated at the sixth generation during the process of establishing the CTS strain according to fasting blood glucose levels; one with euglycaemia (fasting blood glucose: approximately 5.6 mmol/l [eventually became the NOD strain]) and the other with slight hyperglycaemia (fasting blood glucose: approximately 8.3 mmol/l [eventually became the NON strain]). One female mouse in the former line spontaneously developed diabetes at the 20th generation and the inbred NOD strain was established by selective breeding for diabetes. From the latter line the inbred non-obese non-diabetic (NON) strain was established.

In order to further characterise the MHC-linked diabetogenic gene of the NOD mouse, we have analysed the MHC of the NOD mouse using monoclonal antibodies to class I and class II MHC antigens and compared it with its sister (NON, CTS) strains. Schematised in Figure 1 is the genealogy of the three strains and for reference the general "conclusions" from the present study concerning the MHC typing of these 3 strains.

Materials and methods

Mice

The nucleus of the NOD, NON and CTS mice were originally obtained from the colony of Dr. Makino at the Aburahi Laboratories, Shionogi and Company, Ltd., (Shiga, Japan), bred by brother-sister mating at the animal resources center and the radioisotope center of Kyoto University (Kyoto, Japan), and raised in our animal facility at the Joslin Diabetes Center. Female mice at 4 to 16 months of age were used in the present study. The NOD, NON and CTS mice at the time of study were at 39th, 43rd and 84th generation respectively.

Antibodies

Monoclonal antibodies to class I MHC antigens were purchased from Litton Bionetics (Charleston, SC, USA). Monoclonal antibodies to class II MHC antigens used in the present study were 10-2-16 [7], 39B [8], 39F [8], 40A [8], 3JP [2], 17-227 [2], 25-9-17 [2], MKD6 [2], 14-4-4 [2] and M5/114 [2]. Monoclonal antibody AF6-120 was provided by Dr. D. McKean (Mayo Clinic, Rochester, Minn, USA).

MHC typing

The expression of MHC antigens was determined by indirect immunofluorescence. Splenocytes were isolated from NOD, NON and CTS mice with Ficoll-400 (Lympholyte-M, Cederlane; Ontario, Canada), incubated with monoclonal antibodies to class I and class II MHC antigens for 45 min at 4°C and washed three times with RPMI 1640 media. Then the cells were stained with fluorescein isothiocyanate (FITC) conjugated F(ab')₂ goat anti-mouse IgG (gamma and light chain specific, Tago, Inc., Burlingame, Calif, USA) for class I or FITC conjugated F(ab')₂ goat anti-mouse IgG (Fc fragment gamma chain specific, Cappel, Malvern, Penn, USA) for class II MHC antigen. The percentage of fluorescence positive cells were determined by Coulter EPICS flow cytometer analysis. Nonspecific monoclonal antibody (P3X63) was used as a negative control and fluorescence with this antibody was subtracted.

Statistical analysis

Statistical analysis was carried out by binomial distribution.

Results

Table 1 summarises the reactivity of splenocytes from NOD, NON and CTS mice with monoclonal antibodies to class I and class II molecules. NOD splenocytes reacted with monoclonal antibody 31-3-4S (anti-K^d) and 28-14-8S (anti-D^b). In contrast NON splenocytes reacted with monoclonal antibody 28-13-3S (anti-K^b) and 16-1-11N (anti-K^k), suggesting that the H-2K molecules of the NON mouse is different from conventional K^b or K^k. At the D region, the NON mice showed the same reactivity with anti H-2D monoclonals as NOD mice.

Table 1. Reactivity of splenocytes from NOD, NON and CTS mice with anti-class I and class II monoclonal antibodies

	Monoclonal antibody	MHC region	NOD	NON	CTS	C57BL/6 (H-2 ^b)	BALB/c (H-2 ^d)	C3H/He (H-2 ^k)
Class I	28-13-3S ^a	K	0 ^b	38	0	39	0	0
	31-3-4S	K	31	6	60	2	26	0
	16-1-11N	K	2	40	60	0	0	44
	28-14-8S	D	46	31	2	30	33	0
	34-4-21S	D	0	2	0	0	42	0
	12-2-2S	D, K	0	0	6	0	0	30
Class II	10-2-16	I-A	44	40	40	1	0	40
	39B	I-A	29	3	34	1	0	33
	40A	I-A	42	1	52	1	0	39
	39F	I-A	6	1	1	1	1	32
	3JP	I-A	0	47	0	38	0	NT ^c
	AF6-120	I-A	1	0	0	26	0	NT
	17-227	I-A	1	15	0	51	1	NT
	25-9-17	I-A	0	0	0	51	47	NT
	MKD6	I-A	0	23	0	0	43	NT
	M5/114	I-A, -E	0	33	0	47	48	NT
	14-4-4	I-E	0	33	0	0	45	NT

^a Specificities of monoclonal antibodies are as follows: Class I (P: primary specificity, C: cross-reactions) 28-13-3S, P: H-2K^b, C: H-2^f; 31-3-4S, P: H-2K^d, C: none; 16-1-11N, P: H-2K^k, C: H-2K^q, H-2^p, H-2^r; 28-14-8S, P: H-2D^b, C: H-2L^d, H-2D^q and/or H-2L^q; 34-4-21S, P: H-2D^d, C: none; 12-2-2S, P: H-2D^k, H-2K^k, C: H-2K^q, H-2^p, H-2^r. Class II: 10-2-16, I-A^{k,lr,s}; 39B, I-A^{k,lr,u}; 40A, I-A^{k,lr,u}; 39F, I-A^k; 3JP, I-A^{b,kl,p,q,r,s,u,v}; AF6-120, I-A^b; 17-227, I-A^{b,k}; 25-9-17, I-A^{b,d}; MKD6, I-A^d; M5/114, I-A^{b,d}, I-E^{d,k}; 14-4-4, I-E^{k,d,p,r}, but not strains lacking surface I-E such as H-2^{b,f,q,s}. ^b % fluorescent positive cells with Coulter EPICS fluorescent cytometer analysis. Non-specific monoclonal antibody (P3X63) fluorescence subtracted. ^c Not tested, NT. NOD splenocytes were also studied using other monoclonal antibodies, and were negative with anti-K^k monoclonals (16-3-1N, 16-3-22N), anti-D^d monoclonal (34-4-20S), positive with anti-K^d monoclonal (34-1-2S) and anti-D^b monoclonals (27-11-13S, 28-8-6S, 28-11-5S), consistent with NOD's class I MHC as K^dD^b.

CTS splenocytes reacted with monoclonal antibody 31-3-4S (anti-K^d) and 16-1-11N (anti-K^k), suggesting that H-2K molecules of the CTS mouse are also different from conventional K^d or K^k. CTS splenocytes did not react with the three anti-H-2D monoclonal antibodies tested (k,d,b). Thus at both H-2K and H-2D CTS mice differ from NOD mice.

Three monoclonal antibodies (10-2-16, 39B and 40A) reacted with the class II molecules of the NOD mouse. Among these monoclonals, 39B and 40A reacted with I-A of the NOD mouse but not with the NON mouse, while 10-2-16 reacted with the NON and CTS mouse as well as the NOD mouse.

Our prior studies have demonstrated that NOD mice do not express I-E molecules and lack messenger RNA for I-E alpha [2]. NOD splenocytes did not react with anti I-E monoclonal antibody 14-4-4, consistent with our prior studies while the NON splenocytes did react. Monoclonal antibody 14-4-4 reacts with all H-2 haplotypes expressing surface I-E molecules [2], but does not react with strains lacking surface I-E molecules, such as H-2^{b,f,q,s} [2]. Reactivity of the CTS splenocytes with anti-class II MHC monoclonal antibodies was identical to the NOD mouse, reacting with the same 3 of 10 monoclonal antibodies to I-A molecules and not with anti-I-E monoclonal 14-4-4.

In order to analyse the inheritance of diabetes relative to the inheritance of the NOD's class II MHC, NON mice were crossed with NOD mice. None of the F1 animals developed diabetes, confirming our previous finding of recessive contribution of MHC-linked

diabetogenic gene. In backcross generation [NOD × F1(NOD × NON)] all the diabetic backcrosses analysed (6/6) were positive with 40A but negative with MKD6, indicating homozygosity for the NOD's unique I-A in these animals and confirming MHC-linkage of the diabetogenic gene in the NOD mouse ($p < 0.02$). Homozygosity for the NOD's I-A in these animals was also confirmed by RFLP analysis with I-A beta probe.

In that splenocytes from NOD and CTS mice gave identical reactivity with the anti-class II monoclonals studied, we evaluated I-A restriction fragment polymorphism of NOD and CTS mice. Using an I-A-alpha genomic probe with restriction endonuclease HindIII, the CTS mouse had a 13kb band identical to the NOD mouse while the NON mouse had a 2.7kb band. Using an I-A-beta genomic probe with restriction endonuclease BamHI, the CTS mouse had a 9.5kb band as did the NOD mouse, while the NON mouse had a 2.2kb band.

Discussion

In both man and the BB rat, the development of insulin-dependent diabetes is linked with the MHC [9, 10]. We have recently reported that insulin-dependent diabetes of the NOD mouse is also determined by an unknown gene (or genes) tightly linked with NOD mouse's MHC [2]. Thus an MHC linked diabetogenic

gene is a common feature of insulin-dependent diabetes regardless of the species. Precise characterisation of the MHC in the NOD mouse is extremely important for the analysis and localisation of diabetogenic gene(s). In particular, sequence studies by H. Acha-Orbea and H.O. McDevitt [12] indicate that the NOD's I-A beta chain differs from all characterised murine strains, suggesting it may contribute to the development of diabetes.

The class I MHC of the NOD mouse was consistent with K^d and D^b, confirming a previous observation by Dr. K. Moriwaki and ourselves (Makino and Hattori) [11] with conventional allo-antisera. At the D region NOD and NON mice had an identical pattern of reactivity with monoclonals studied while at the K region, NON mice reacted with both anti-K^b and K^k monoclonal antibodies. CTS mice have different class I MHC from the NOD mouse, at the K region (reacting with both anti-K^d and K^k monoclonals) and at the D region (reacting with none of the monoclonals tested [k,d,b]).

In our initial studies [2] we could not find any monoclonal antibodies reacting with class II molecules of the NOD mouse. Using a microcytotoxicity assay with a wider panel of allo-antisera and monoclonal antibodies in collaboration with Dr. D.B. Murphy, we found three monoclonal antibodies (10-2-16, 39B and 40A) reacting with I-A molecules of the NOD mouse. Monoclonal 10-2-16 reacts with NON, CTS and NOD mice. In contrast, monoclonals 39B and 40A reacted with NOD but not with NON mice. In fact, using monoclonal 40A (reacts with NOD but not NON mice) and monoclonal MKD6 (reacts with NON but not NOD mice) for analysis, linkage of the MHC to diabetes could clearly be followed in crosses of NOD mice with NON mice. All diabetic backcross animals (6/6) reacted with 40A but not MKD6.

The primary specificity of monoclonal antibodies 10-2-16, 39B and 40A is reported to be I-A^k [7, 8]. Monoclonal antibody 10-2-16 cross-reacts with I-A^{f,r,s} [7], while 39B and 40A with I-A^{f,r,u} [8]. NOD splenocytes, however, did not react with other monoclonal antibodies with specificity of I-A^k (39F, 3JP, 17-227) or I-A^{f,r,s,u} (3JP). Therefore, the I-A molecules of the NOD mouse are unique, not fitting any known MHC specificity pattern. This data on monoclonal anti-I-A reactivity of the NOD mouse is consistent with recent cDNA sequence data by H. Acha-Orbea and H.O. McDevitt [12], in which first external domain of I-A beta chain was unique for the NOD mouse while other parts of the I-A beta chain and I-A alpha chain were identical to H-2^d haplotype.

I-A molecules of the NON mouse are also unique, not fitting any known MHC specificity pattern, but clearly different from the NOD mouse. I-A molecules of the NON mouse reacted with 3JP (I-A^{b,f,k,p,q,s,u,v}), 17-227 (I-A^{b,k}) and MKD6 (I-A^d) as well as 10-2-16 (I-A^{k,f,r,s}), but not with 39B or 40A (I-A^{k,f,r,u}). In addition,

NON mice express I-E molecules in contrast to the NOD mouse which lacks surface I-E molecules and mRNA for I-E alpha [2].

In contrast to NON mice, class II molecules of the CTS mouse showed the same reactivity as the NOD mouse with the monoclonal antibodies studied (Table 1). In addition, using an I-A alpha genomic probe with restriction endonuclease HindIII or an I-A beta genomic probe with BamHI, the CTS mouse was also identical to the NOD mouse while the NON mouse was different from the NOD mouse. These data suggest that class II MHC of the CTS mouse may be the same as the NOD mouse though class I genes differ.

Although breeding studies by our [2, 3] and other [4, 5] laboratories suggest that the unique MHC of the NOD mouse is diabetogenic, exact localisation of MHC-linked diabetogenic genes is still unknown. Recent breeding studies in crosses of NOD with I-E expressing C57BL/6 transgenic mice [13] suggest that an MHC-linked recessive influence on insulinitis may relate I-E alpha gene expression, but involvement of other MHC-linked diabetogenic genes, including I-A was also suggested. From the sequence studies it has been suggested that the unique I-A beta of the NOD is diabetogenic, but no recombinants between this I-A gene and other MHC genes have yet been defined to allow definitive analysis. Since the CTS mouse may be a naturally occurring recombinant strain with class II MHC of the NOD type and class I MHC of non-NOD type, the CTS mouse provides a new strain for studying the diabetogenic region in breeding studies.

The development of diabetes of the NOD mouse is controlled by multiple (probably three) recessive genes [2-5]. Therefore, CTS mice may be free of diabetes even if they share the potentially diabetogenic class II MHC of the NOD mouse. As indicated by the current study, DNA sequencing of the I-A MHC of the CTS mouse and breeding studies in crosses of the NOD with the CTS mouse should provide more information on the actual localisation of the diabetogenic histocompatibility gene of the NOD mouse.

Acknowledgements. We thank Dr. D.B. Murphy and Dr. C.A. Jane-way at Yale University for helping us to find monoclonal antibody 10-2-16 and valuable criticisms. Our thanks are extended to Dr. L. H. Glimcher, Dr. K. Ozato and Dr. C.S. David for the gift of class I and class II monoclonal antibodies, K. George and D. Watt for their technical assistance and P. Cronin-Sevigny for secretarial assistance.

This work was supported in part by grants from the National Institutes of Health (DK33641, DERC 1 P30-DK36837) and the Juvenile Diabetes Foundation (185292). H. Ikegami is a recipient of a Postdoctoral Fellowship Award from the Juvenile Diabetes Foundation.

References

1. Makino S, Kunitomo K, Muraoka Y, Mizushima Y, Katagiri K, Tochino Y (1980) Breeding of non-obese diabetic strain of mice. *Exp Animal* 29: 1-13

2. Hattori M, Buse JB, Jackson RA, Glimcher L, Dorf ME, Minami M, Makino S, Moriwaki K, Kuzuya H, Imura H, Strauss WM, Seidman JG, Eisenbarth GS (1986) The NOD mouse: recessive diabetogenic gene in the major histocompatibility complex. *Science* 231: 733-735
3. Ikegami H, Jackson RA, Makino S, Watt DE, Eisenbarth GS, Hattori M (1986) Homozygosity for two genes (H-2: chromosome 17 and Thy-1: chromosome 9) linked to development of Type I diabetes of the NOD mouse. *Clin Res* 34: 683A
4. Wicker LS, Miller BJ, Coker LZ, McNally SE, Scott S, Mullen Y, Appel MC (1987) Genetic control of diabetes and insulinitis in the non-obese diabetic (NOD) mouse. *J Exp Med* 165: 1639-1654
5. Prochazka M, Leiter EH, Serreze DV, Coleman DL (1987) Three recessive loci required for insulin dependent diabetes in non-obese diabetic mice. *Science* 237: 286-289
6. Ohotori H, Yoshida T, Inuta T (1968) "Small eyes and cataract", a new dominant mutation in the mouse. *Exp Animal* 17: 91-96
7. Oi VT, Jones PP, Goding JW, Herzenberg LA (1978) Properties of monoclonal antibodies to mouse Ig allotypes, H-2 and Ia antigens. *Curr Top Microbiol Immunol* 81: 115-129
8. Pierres M, Devaux C, Dosseto M, Marchetto S (1981) Clonal analysis of B- and T-cell responses to Ia antigens. I. Topology of epitope regions on I-A^k and I-E^k molecules analyzed with 35 monoclonal alloantibodies. *Immunogenetics* 14: 481-495
9. Wolf E, Spencer KM, Cudworth AG (1983) The genetic susceptibility to Type I (insulin dependent) diabetes: analysis of the HLA-DR association. *Diabetologia* 24: 224-230
10. Colle E, Guttman RD, Seemayer T (1981) Spontaneous diabetes mellitus syndrome in the rat. I. Association with the major histocompatibility complex. *J Exp Med* 154: 1237-1241
11. Moriwaki K, Sagai T, Suzuki K, Makino S, Hattori M (1988) Genetic background of disease model mice: (1) H-2 haplotype and chromosome C-banding patterns in NOD and NON strains. *Exp Animal* (in press)
12. Acha-Orbea H, McDevitt HO (1987) The first external domain of the nonobese diabetic mouse class II I-A beta chain is unique. *Proc Natl Acad Sci USA* 84: 2435-2439
13. Nishimoto H, Kikutani H, Yamamura K, Kishimoto T (1987) Prevention of autoimmune insulinitis by expression of I-E molecules in NOD mice. *Nature* 328: 432-434

Received: 10 November 1987
and in revised form: 28 January 1988

Dr. H. Ikegami
Joslin Diabetes Center
1 Joslin Place
Boston, Massachusetts 02215
USA